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CHATTERJEE, DEB K.
Appl. No. 09/229,173

extensions of time are specifically requested and the fee should also be charged to our deposit account.

Amendments

In the Claims:

Please cancel claims 4 and 39 without prejudice or disclaimer.

Please replace pending claims 1, 5, 6, 36-38, 40 and 44 with the following claims

1, 5, 6, 36-38, 40 and 44:

DL
Gul
GJ

1. (Once amended) A *Thermotoga maritima* (Tma) DNA polymerase mutant which is modified at least two ways selected from the group consisting of:

- (a) a mutation in the 3'-5' exonuclease domain of said polymerase to reduce or eliminate the 3'→5' exonuclease activity of the polymerase;
- (b) a mutation in the 5'-3' exonuclease domain of said polymerase to reduce or eliminate the 5'→3' exonuclease activity of the polymerase; and
- (c) a mutation in the O-helix of said polymerase to reduce or eliminate discriminatory behavior against a dideoxynucleotide.

D2

5. (Once amended) The DNA polymerase of claim 1, wherein said O-helix is defined as RXXXXXXXFXXXXYX (SEQ ID NO:1), wherein X is any amino acid.

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D2

6. (Once amended) The *Tma* DNA polymerase as claimed in claim 1, wherein said mutation in the O-helix is a Phe⁷³⁰→Tyr⁷³⁰ substitution.

D3

36. (Once amended) A method of sequencing a DNA molecule, comprising:
- (a) hybridizing a primer to a first DNA molecule;
 - (b) contacting said DNA molecule of step (a) with deoxyribonucleoside triphosphates, the DNA polymerase of claim 1, and a terminator nucleotide;
 - (c) incubating the mixture of step (b) under conditions sufficient to synthesize a random population of DNA molecules complementary to said first DNA molecule, wherein said synthesized DNA molecules are shorter in length than said first DNA molecule and wherein said synthesized DNA molecules comprise a terminator nucleotide at their 3' termini; and
 - (d) separating said synthesized DNA molecules by size so that at least a part of the nucleotide sequence of said first DNA molecule can be determined.

D4

37. (Once amended) An isolated DNA molecule encoding a *Thermotoga maritima* (*Tma*) DNA polymerase mutant which is modified at least two ways selected from the group consisting of:

- (a) a mutation in the 3'-5' exonuclease domain of said polymerase to reduce or eliminate the 3' 5' exonuclease activity of the polymerase;
- (b) a mutation in the 5'-3' exonuclease domain of said polymerase to reduce or eliminate the 5' 3' exonuclease activity of the polymerase; and
- (c) a mutation in the O-helix of said polymerase to reduce or eliminate discriminatory behavior against a dideoxynucleotide.

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D4
38. (Once amended) A mutant *Tma* DNA polymerase having a mutation in the O-helix, resulting in said DNA polymerase becoming non-discriminating against dideoxynucleotides, or a fragment of said mutant DNA polymerase said fragment having polymerase activity.

D5
40. (Once amended) The mutant *Tma* DNA polymerase of claim 38, wherein said O-helix is defined as RXXXKXXXFXXXYX, wherein X is any amino acid.

D6
44. (Once amended) A method of sequencing a DNA molecule, comprising:
(a) hybridizing a primer to a first DNA molecule;
(b) contacting said DNA molecule of step (a) with dextrynucleoside triphosphates, a DNA polymerase of claim 38, and a terminator nucleotide;
(c) incubating the mixture of step (b) under conditions sufficient to synthesize a random population of DNA molecules complementary to said first DNA molecule, wherein said synthesized DNA molecules are shorter in length than said first DNA molecule and wherein said synthesized DNA molecules comprise a terminator nucleotide at their 3' termini; and
(d) separating said synthesized DNA molecules by size so that at least a part of the nucleotide sequence of said first DNA molecule can be determined.
